SEQUENCE LISTING

<110> SOCIETE DE CONSEILS DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES (S.C.R.A.S.)

<120> Process for preparing recombinant heterocarpine

<130> RS 331 PCT

<150> FR 02/15563

<151> 2002-12-10

<160> 14

<170> PatentIn version 3.1

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<212> PRT

<213> Pilocarpus Heterophyllus (peptide fragment)

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<210> 2

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<213> Pilocarpus Heterophyllus (peptide fragment)

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1 5 10

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Pro Glu Ser Glu Ser Tyr
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<213> Artificial Sequence

<220>

<223> Primer for 5' specific cDNA products

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<210> 5

<211> 38

<212> DNA

<213> Artificial Sequence

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<223> Primer for 3' specific cDNA products

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cggtatggac gcggctattg ctgatggtgt tgatgtaa 38

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<211> 1675

<212> DNA

<213> Pilocarpus Heterophyllus (5' cDNA fragment of the cDNA encoding for heterocarpine)

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tctaggaatg gtgttcttca tttattcctt ttcgttcttg catggcttct gttggcggct 180

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aagtctacaa agccaaccaa attaaatcgc cgtcgatcct caccacttct tgtatactct 360

tacgacaatg ctgctcatgg tttcagtgca gttttatctc aacaggaact tgaaactcta 420

aaaaagtctc caggtttcgt ctcagtttat gccgataaga cagcgacact tgacaccacc 480

catacacctg aatttctctc cctgaatact gccaacgggt tgtggcctgc ttcaaagtat 540

ggtgaagata taattgttgg tgttattgac agcggtgtct ggccggagag tgaaagttat 600

aatgatgatg gtatgggcgc tattccaagc agatggaagg gagaatgtga agctggacaa 660

gagttcaatt cctccatgtg caactcaaag cttattggag ctagatattt cgataagggt 720

atcattgcgg caaatcctgg gattaacatt agcatgaaat ctgccagaga tactatgggg 780

catgggactc acacatcctc cacagttgct gggaattatg tggatggcgt ttcattcttt 840

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gcatcgaaca ttcggggagc catatttgtt tcagattatg atgctgaatt atttgaactt 1440

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tacgccagca atgatgtgaa acctaaggca agcatcaagt tccaacaaac tgttctgggc 1560

acaaagcctg caccagccgt ggctttctat acttctagag gtccgtcacc gagctatcca 1620

ggcatcttaa agccagatat aatggcccct gggtcactag tttttgctgc ttgga 1675

<210> 7

<211> 1689

<212> DNA

<213> Pilocarpus Heterophyllus (3' cDNA fragment of the cDNA encoding for heterocarpine)

<400> 7

cggtatggac gcggctattg ctgatggtgt tgatgtaatt tcaatatcaa tgggatttga 60

tgagaccccg ttgtatgaag atcctatagc aattgcctca ttcgctgcta cagagaaggg 120

cgtagtggtc tcatcttcag caggaaatgc agggccagcg ctagggagct tgcacaatgg 180

aatcccatgg acgttaactg ttgcagctgg aaccattgac cgttcatttg caggcactat 240

aactcttggg agtggggaaa ccatcattgg atggacaatg ttcccagcca gtgcttatgt 300

agcagacttg ccactgcttt ataacaagac ttactctgca tgcaactcaa ctcgattatt 360

atctcaactc cgaactgacg ccatcatcgt atgcgaagaa gctgaagatt cggtatctga 420

gcaaatatct gttgtcagtg catcgaacat tcggggagcc atatttgttt cagattatga 480

tgctgaatta tttgaacttg gtggtgtgac tattcctggt gtcgtgatta gcaccaagga 540

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gactacagca aatcccttgg ataacacact aaatccaatc cgggacaatg gtctaatcaa 960

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gaacttcacc caaaaccaaa tcctgtccat tacaagatca aaccgttaca gctgctccac 1140

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gagccctatt acagtgtcac cttccatgag taactttgtg tttatgggta cacaataatt 1500

gataaaaatt tgttctgatc acaactgtgg gaataatcga cgtttatgaa cccagaataa 1560

gttgtttggt cgtcttcaac attatcataa aggacttgaa tcatgtgtgt tgattttctg 1620

caaaaaaaaa aaaaaaaaaa aagtactctg cgttgatacc actgcttgcc ctatagtgag 1680

tcgtattag 1689

<210> 8

<211> 2630

<212> DNA

<213> Pilocarpus Heterophyllus (cDNA encoding for heterocarpine)

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aatgatgatg gtatgggcgc tattccaagc agatggaagg gagaatgtga agctggacaa 660
gagttcaatt cctccatgtg caactcaaag cttattggag ctagatattt cgataagggt 720
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catgggactc acacatcctc cacagttgct gggaattatg tggatggcgt ttcattcttt 840
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ccttccatga gtaactttgt gtttatgggt acacaataat tgataaaaat ttgttctgat 2460

cacaactgtg ggaataatcg acgtttatga acccagaata agttgtttgg tcgtcttcaa 2520

cattatcata aaggacttga atcatgtgtg ttgattttct gcaaaaaaaa aaaaaaaaaa 2580

aaagtactct gcgttgatac cactgcttgc cctatagtga gtcgtattag 2630

<210> 9

<211> 2325

<212> DNA

<213> Pilocarpus Heterophyllus (encoding part of cDNA encoding for heterocarpine)

<400> 9

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gcggctctcc atgctaactc aagttcggat gagagatcaa catatatagt tcatatggac 120

aagacccata tgcccaaaac cttctctagc ccccaccatt ggtactcttc ggtcgttcga 180

tccctcaagt ctacaaagcc aaccaaatta aatcgccgtc gatcctcacc acttcttgta 240

tactcttacg acaatgctgc tcatggtttc agtgcagttt tatctcaaca ggaacttgaa 300

actctaaaaa agtctccagg tttcgtctca gtttatgccg ataagacagc gacacttgac 360

accacccata cacctgaatt tctctccctg aatactgcca acgggttgtg gcctgcttca 420

aagtatggtg aagatataat tgttggtgtt attgacagcg gtgtctggcc ggagagtgaa 480

agttataatg atgatggtat gggcgctatt ccaagcagat ggaagggaga atgtgaagct 540

ggacaagagt tcaattcctc catgtgcaac tcaaagctta ttggagctag atatttcgat 600

aagggtatca ttgcggcaaa tcctgggatt aacattagca tgaaatctgc cagagatact 660

atggggcatg ggactcacac atcctccaca gttgctggga attatgtgga tggcgtttca 720

ttctttggct atgctaaagg tacagcaaaa ggagtggcac cacgggcgag agtggctatg 780

tacaaggtca tttttgacga agggcgctat gcatctgatg ttcttgccgg tatggacgcg 840

gctattgctg atggtgttga tgtaatttca atatcaatgg gatttgatga gaccccgttg 900

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gtgtcacctt ccatgagtaa ctttgtgttt atgggtacac aataa 2325

<210> 10

<211> 774

<212> PRT

<213> Pilocarpus Heterophyllus (heterocarpine)

<400> 10

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Trp Leu Leu Leu Ala Ala Leu His Ala Asn Ser Ser Ser Asp Glu Arg
20 25 30

Ser Thr Tyr Ile Val His Met Asp Lys Thr His Met Pro Lys Thr Phe
35 40 45

Ser Ser Pro His His Trp Tyr Ser Ser Val Val Arg Ser Leu Lys Ser
50 55 60

Thr Lys Pro Thr Lys Leu Asn Arg Arg Arg Ser Ser Pro Leu Leu Val
65 70 75 80

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Tyr Ser Tyr Asp Asn Ala Ala His Gly Phe Ser Ala Val Leu Ser Gln
                85                  90                  95

Gln Glu Leu Glu Thr Leu Lys Lys Ser Pro Gly Phe Val Ser Val Tyr
            100                 105                 110

Ala Asp Lys Thr Ala Thr Leu Asp Thr Thr His Thr Pro Glu Phe Leu
        115                 120                 125

Ser Leu Asn Thr Ala Asn Gly Leu Trp Pro Ala Ser Lys Tyr Gly Glu
    130                 135                 140

Asp Ile Ile Val Gly Val Ile Asp Ser Gly Val Trp Pro Glu Ser Glu
145                 150                 155                 160

Ser Tyr Asn Asp Asp Gly Met Gly Ala Ile Pro Ser Arg Trp Lys Gly
                165                 170                 175

Glu Cys Glu Ala Gly Gln Glu Phe Asn Ser Ser Met Cys Asn Ser Lys
            180                 185                 190

Leu Ile Gly Ala Arg Tyr Phe Asp Lys Gly Ile Ile Ala Ala Asn Pro
        195                 200                 205

Gly Ile Asn Ile Ser Met Lys Ser Ala Arg Asp Thr Met Gly His Gly
    210                 215                 220

Thr His Thr Ser Ser Thr Val Ala Gly Asn Tyr Val Asp Gly Val Ser
225                 230                 235                 240

Phe Phe Gly Tyr Ala Lys Gly Thr Ala Lys Gly Val Ala Pro Arg Ala
                245                 250                 255

Arg Val Ala Met Tyr Lys Val Ile Phe Asp Glu Gly Arg Tyr Ala Ser
            260                 265                 270

Asp Val Leu Ala Gly Met Asp Ala Ala Ile Ala Asp Gly Val Asp Val
        275                 280                 285

Ile Ser Ile Ser Met Gly Phe Asp Glu Thr Pro Leu Tyr Glu Asp Pro
    290                 295                 300

Ile Ala Ile Ala Ser Phe Ala Ala Thr Glu Lys Gly Val Val Val Ser
305                 310                 315                 320

Ser Ser Ala Gly Asn Ala Gly Pro Ala Leu Gly Ser Leu His Asn Gly
                325                 330                 335

Ile Pro Trp Thr Leu Thr Val Ala Ala Gly Thr Ile Asp Arg Ser Phe
            340                 345                 350
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Ala Gly Thr Ile Thr Leu Gly Ser Gly Glu Thr Ile Ile Gly Trp Thr
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Met Phe Pro Ala Ser Ala Tyr Val Ala Asp Leu Pro Leu Leu Tyr Asn
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Lys Thr Tyr Ser Ala Cys Asn Ser Thr Arg Leu Leu Ser Gln Leu Arg
385                 390                 395                 400

Thr Asp Ala Ile Ile Val Cys Glu Glu Ala Glu Asp Ser Val Ser Glu
                405                 410                 415

Gln Ile Ser Val Val Ser Ala Ser Asn Ile Arg Gly Ala Ile Phe Val
            420                 425                 430

Ser Asp Tyr Asp Ala Glu Leu Phe Glu Leu Gly Gly Val Thr Ile Pro
        435                 440                 445

Gly Val Val Ile Ser Thr Lys Asp Ala Pro Ala Val Ile Ser Tyr Ala
    450                 455                 460

Ser Asn Asp Val Lys Pro Lys Ala Ser Ile Lys Phe Gln Gln Thr Val
465                 470                 475                 480

Leu Gly Thr Lys Pro Ala Pro Ala Val Ala Phe Tyr Thr Ser Arg Gly
                485                 490                 495

Pro Ser Pro Ser Tyr Pro Gly Ile Leu Lys Pro Asp Ile Met Ala Pro
            500                 505                 510

Gly Ser Leu Val Phe Ala Ala Trp Ile Pro Asn Thr Ala Thr Ala Gln
        515                 520                 525

Ile Gly Leu Asn Thr Leu Leu Thr Ser Glu Tyr Asn Met Val Ser Gly
    530                 535                 540

Thr Ser Met Ala Cys Pro His Ala Ala Gly Val Ala Ala Leu Leu Lys
545                 550                 555                 560

Gly Ala His Pro Glu Trp Ser Ala Ala Ala Ile Arg Ser Ala Met Met
                565                 570                 575

Thr Thr Ala Asn Pro Leu Asp Asn Thr Leu Asn Pro Ile Arg Asp Asn
            580                 585                 590

Gly Leu Ile Asn Phe Thr Ser Ala Ser Pro Leu Ala Met Gly Ala Gly
        595                 600                 605

Gln Val Asp Pro Asn Arg Ala Leu Asp Pro Gly Leu Ile Tyr Glu Thr
    610                 615                 620
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Thr Pro Gln Asp Tyr Val Ser Leu Leu Cys Thr Leu Asn Phe Thr Gln
625 630 635 640

Asn Gln Ile Leu Ser Ile Thr Arg Ser Asn Arg Tyr Ser Cys Ser Thr
645 650 655

Pro Asn Pro Asp Leu Asn Tyr Pro Ser Phe Ile Thr Leu His Tyr Asn
660 665 670

Thr Asn Ala Thr Phe Val Gln Thr Phe His Arg Thr Val Thr Asn Val
675 680 685

Gly Gly Ser Ala Thr Thr Tyr Lys Ala Lys Ile Thr Ala Pro Leu Gly
690 695 700

Ser Val Val Ser Val Ser Pro Asp Thr Leu Ala Phe Arg Lys Gln Tyr
705 710 715 720

Glu Gln Gln Ser Tyr Glu Leu Thr Ile Glu Tyr Lys Pro Asp Gly Glu
725 730 735

Glu Thr Val Ser Phe Gly Glu Leu Val Trp Ile Glu Glu Asn Gly Asn
740 745 750

His Thr Val Arg Ser Pro Ile Thr Val Ser Pro Ser Met Ser Asn Phe
755 760 765

Val Phe Met Gly Thr Gln
770

<210> 11

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer for 5' specific cDNA products

<400> 11
gggggatccg aggtctagga atggtgttct tca 33

<210> 12

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer for 3' specific cDNA products

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<210> 13

<211> 2338

<212> DNA

<213> Artificial Sequence

<220>

<223> cDNA encoding for heterocarpine having undergone a deletion of the initiation codon and a deletion of the STOP codon

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tctgttggcg gctctccatg ctaactcaag ttcggatgag agatcaacat atatagttca 120

tatggacaag acccatatgc ccaaaacctt ctctagcccc caccattggt actcttcggt 180

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Leu His Leu Phe Leu Phe Val Leu Ala Trp Leu Leu Leu Ala Ala Leu
20 25 30

His Ala Asn Ser Ser Ser Asp Glu Arg Ser Thr Tyr Ile Val His Met
35 40 45

Asp Lys Thr His Met Pro Lys Thr Phe Ser Ser Pro His His Trp Tyr
50 55 60

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Ser Ser Val Val Arg Ser Leu Lys Ser Thr Lys Pro Thr Lys Leu Asn
65                  70                  75                  80

Arg Arg Arg Ser Ser Pro Leu Leu Val Tyr Ser Tyr Asp Asn Ala Ala
                85                  90                  95

His Gly Phe Ser Ala Val Leu Ser Gln Gln Glu Leu Glu Thr Leu Lys
            100                 105                 110

Lys Ser Pro Gly Phe Val Ser Val Tyr Ala Asp Lys Thr Ala Thr Leu
        115                 120                 125

Asp Thr Thr His Thr Pro Glu Phe Leu Ser Leu Asn Thr Ala Asn Gly
    130                 135                 140

Leu Trp Pro Ala Ser Lys Tyr Gly Glu Asp Ile Ile Val Gly Val Ile
145                 150                 155                 160

Asp Ser Gly Val Trp Pro Glu Ser Glu Ser Tyr Asn Asp Asp Gly Met
                165                 170                 175

Gly Ala Ile Pro Ser Arg Trp Lys Gly Glu Cys Glu Ala Gly Gln Glu
            180                 185                 190

Phe Asn Ser Ser Met Cys Asn Ser Lys Leu Ile Gly Ala Arg Tyr Phe
        195                 200                 205

Asp Lys Gly Ile Ile Ala Ala Asn Pro Gly Ile Asn Ile Ser Met Lys
    210                 215                 220

Ser Ala Arg Asp Thr Met Gly His Gly Thr His Thr Ser Ser Thr Val
225                 230                 235                 240

Ala Gly Asn Tyr Val Asp Gly Val Ser Phe Phe Gly Tyr Ala Lys Gly
                245                 250                 255

Thr Ala Lys Gly Val Ala Pro Arg Ala Arg Val Ala Met Tyr Lys Val
            260                 265                 270

Ile Phe Asp Glu Gly Arg Tyr Ala Ser Asp Val Leu Ala Gly Met Asp
        275                 280                 285

Ala Ala Ile Ala Asp Gly Val Asp Val Ile Ser Ile Ser Met Gly Phe
    290                 295                 300

Asp Glu Thr Pro Leu Tyr Glu Asp Pro Ile Ala Ile Ala Ser Phe Ala
305                 310                 315                 320

Ala Thr Glu Lys Gly Val Val Val Ser Ser Ser Ala Gly Asn Ala Gly
                325                 330                 335
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Pro Ala Leu Gly Ser Leu His Asn Gly Ile Pro Trp Thr Leu Thr Val
            340                 345                 350

Ala Ala Gly Thr Ile Asp Arg Ser Phe Ala Gly Thr Ile Thr Leu Gly
        355                 360                 365

Ser Gly Glu Thr Ile Ile Gly Trp Thr Met Phe Pro Ala Ser Ala Tyr
    370                 375                 380

Val Ala Asp Leu Pro Leu Leu Tyr Asn Lys Thr Tyr Ser Ala Cys Asn
385                 390                 395                 400

Ser Thr Arg Leu Leu Ser Gln Leu Arg Thr Asp Ala Ile Ile Val Cys
                405                 410                 415

Glu Glu Ala Glu Asp Ser Val Ser Glu Gln Ile Ser Val Val Ser Ala
            420                 425                 430

Ser Asn Ile Arg Gly Ala Ile Phe Val Ser Asp Tyr Asp Ala Glu Leu
        435                 440                 445

Phe Glu Leu Gly Gly Val Thr Ile Pro Gly Val Val Ile Ser Thr Lys
    450                 455                 460

Asp Ala Pro Ala Val Ile Ser Tyr Ala Ser Asn Asp Val Lys Pro Lys
465                 470                 475                 480

Ala Ser Ile Lys Phe Gln Gln Thr Val Leu Gly Thr Lys Pro Ala Pro
                485                 490                 495

Ala Val Ala Phe Tyr Thr Ser Arg Gly Pro Ser Pro Ser Tyr Pro Gly
            500                 505                 510

Ile Leu Lys Pro Asp Ile Met Ala Pro Gly Ser Leu Val Phe Ala Ala
        515                 520                 525

Trp Ile Pro Asn Thr Ala Thr Ala Gln Ile Gly Leu Asn Thr Leu Leu
    530                 535                 540

Thr Ser Glu Tyr Asn Met Val Ser Gly Thr Ser Met Ala Cys Pro His
545                 550                 555                 560

Ala Ala Gly Val Ala Ala Leu Leu Lys Gly Ala His Pro Glu Trp Ser
                565                 570                 575

Ala Ala Ala Ile Arg Ser Ala Met Met Thr Thr Ala Asn Pro Leu Asp
            580                 585                 590

Asn Thr Leu Asn Pro Ile Arg Asp Asn Gly Leu Ile Asn Phe Thr Ser
        595                 600                 605
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Ala Ser Pro Leu Ala Met Gly Ala Gly Gln Val Asp Pro Asn Arg Ala
    610                 615                 620

Leu Asp Pro Gly Leu Ile Tyr Glu Thr Thr Pro Gln Asp Tyr Val Ser
625                 630                 635                 640

Leu Leu Cys Thr Leu Asn Phe Thr Gln Asn Gln Ile Leu Ser Ile Thr
                645                 650                 655

Arg Ser Asn Arg Tyr Ser Cys Ser Thr Pro Asn Pro Asp Leu Asn Tyr
            660                 665                 670

Pro Ser Phe Ile Thr Leu His Tyr Asn Thr Asn Ala Thr Phe Val Gln
        675                 680                 685

Thr Phe His Arg Thr Val Thr Asn Val Gly Gly Ser Ala Thr Thr Tyr
    690                 695                 700

Lys Ala Lys Ile Thr Ala Pro Leu Gly Ser Val Val Ser Val Ser Pro
705                 710                 715                 720

Asp Thr Leu Ala Phe Arg Lys Gln Tyr Glu Gln Gln Ser Tyr Glu Leu
                725                 730                 735

Thr Ile Glu Tyr Lys Pro Asp Gly Glu Glu Thr Val Ser Phe Gly Glu
            740                 745                 750

Leu Val Trp Ile Glu Glu Asn Gly Asn His Thr Val Arg Ser Pro Ile
        755                 760                 765

Thr Val Ser Pro Ser Met Ser Asn Phe Val Phe Met Gly Thr Gln Leu
    770                 775                 780

Glu His His His His His His His His
785                 790
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